Epic Journeys from Bench to Bedside

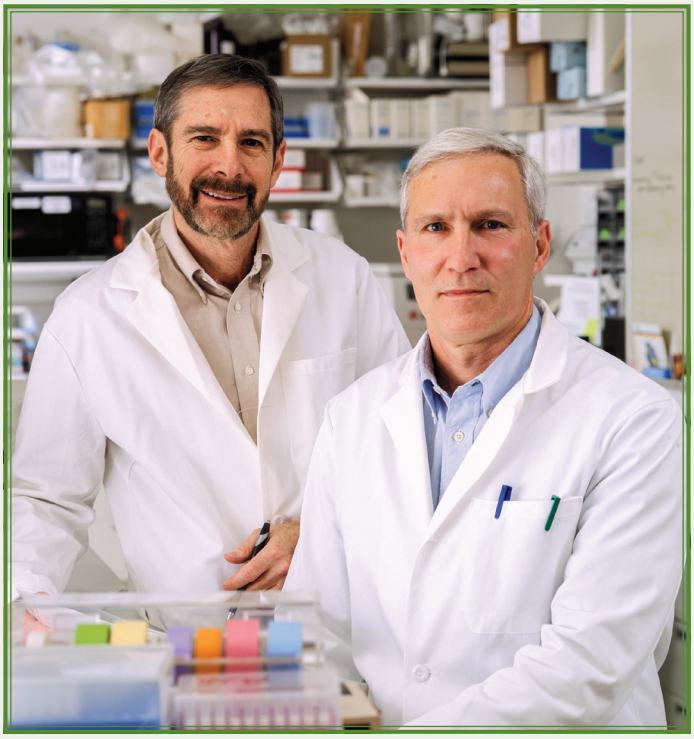
In a world that demands increasingly rapid returns on investment from all sectors of society, basic research can sometimes seem a luxury. Yet, time and again, the value of research that was initiated years or even decades ago is proven in new gains for human health. CCR scientists play a role in all stages of discovery, from basic mechanisms to clinical trials; here are four examples of work pioneered within CCR that has led to currently available drugs for the prevention and treatment of cancer.

An Ounce of Prevention

In the early 1980s, Professor Harald zur Hausen, Ph.D., made the remarkable, Nobel-prize winning discovery that a type of human papilloma virus (HPV)—HPV16—was found in over 50 percent of cervical cancers. This research led to the current understanding that approximately five percent of all cancers are caused by HPV infection. It also led, via CCR, to commercially available vaccines to prevent HPV-induced cancers.

In 1983, zur Hausen gave a lecture on his findings at the NIH in Bethesda. John Schiller, Ph.D., had coincidentally just joined NCI as a Postdoctoral Fellow. "The second lecture I went to at NCI was Harald saying 'Eureka!'."

Schiller had joined the laboratory of Douglas Lowy, M.D., to study the basic mechanisms of viral oncogenesis, thereby beginning a 30-plus year collaboration that now finds them co-Principal Investigators in CCR's Laboratory of Cellular Oncology. They began by studying the individual proteins of an HPV-related virus from cows, bovine papilloma virus type 1 (BPV-1), to understand the viral mechanisms of cellular transformation. After nearly a decade of research on the functions of these proteins, Schiller, Lowy, and their team began a new line of investigation and made a critical discovery about the virus' outer coat protein, L1.



(Photo: R. Baer)

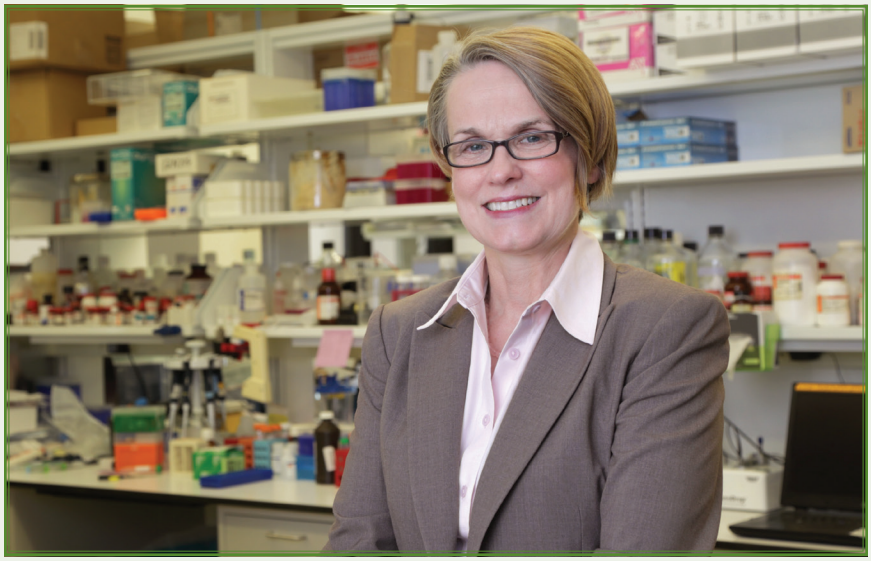
Douglas Lowy, M.D., and John Schiller, Ph.D.

"Previously, people had been making L1 in *E. coli* expression systems, which meant that they were isolating the protein in a denatured state that did not induce antibodies able to prevent virus infection," said Schiller. "When we expressed it in baculovirus-infected insect cells instead, we not only got the native protein but amazingly, 360 copies of this one protein self-assembled into the outer coat of the virus, just as in the authentic virus." Moreover, when these self-assembled virus-like particles (VLPs) were injected into rabbits, they were able to produce very high levels of antibodies that prevented infection by the authentic virus. These were the class of antibodies that are often the cornerstone of preventative vaccines.

The scientists had to overcome a further hurdle in translating this result from bovine to human papilloma virus and setting the stage for a vaccine. At first, they could not extend their findings to HPV16, because its L1 self-assembled very poorly. "That meant that either these two related viruses were acting very differently, or it meant that the HPV16 we were working with was a mutant," said Schiller. The latter possibility made sense because the widely used clone of HPV16 had been isolated from a human cancer, and cancers usually have extensive genomic changes. So the team found a source of HPV16 genomes from normal productive HPV16 lesions—not cancers—and showed that its L1 efficiently self-assembled. A single amino acid change was found to be responsible for the difference in self-assembling properties.

"That really set the stage for bringing companies in," said Schiller. "But, when we first approached the major vaccine manufacturers, there was extreme skepticism that a vaccine against sexually transmitted infections could work at all. There were no examples, despite considerable efforts. Two companies took a leap of faith: Merck and a then small local company, MedImmune."

MedImmune sold its interest in the VLP strategy to GlaxoSmithKline Biologicals (GSK) after successful phase 1 trials. Today, GSK manufactures Cervarix, which contains the L1 VLPs of HPV16 and HPV18, and Merck manufactures Gardasil, which additionally contains L1 VLPs from HPV6 and HPV11, thereby targeting both cancer and genital warts. The U.S. Centers for Disease Control and Prevention recommends that all preteens and previously unvaccinated teens and young adults be administered the vaccine.



(Photo: B. Branson)

Susan Bates, M.D.

NCI's involvement did not end with a handoff to big companies for the clinical trials necessary to obtain regulatory approval. Simultaneously, NCI decided to sponsor its own trial, led by Allan Hildesheim, Ph.D., Chief of the Division of Cancer Epidemiology and Genetics's Infections and Immunoepidemiology Branch at NCI.

"We have preliminary evidence that even a single dose of the vaccine gives very strong protection over four years," said Schiller. "Now we are deciding whether to test, in a dose randomized trial, the efficacy of a single dose, which is unheard of for a vaccine based on only one protein."

"I came to the lab to find out how viruses replicate and transform cells," concluded Schiller. "It was only years later that Doug and I decided to take a crack at this vaccine. It was a real departure for us, but it paid off." (See "CCR Researchers Awarded the National Medal of Technology and Innovation," *CCR connections* Vol. 8, No. 2)

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A Pound of Cure

Susan Bates, M.D., Senior Investigator in CCR's Developmental Therapeutics Branch, started out studying the compound that would become Istodax (romidepsin) because she was interested in multidrug resistance mediated by the cellular pump, P-glycoprotein (PGP), in the 1990s.

Working with the NCI-60 Drug Screen's cell line panel, a highly characterized set of cancer cells whose differential response to drugs is used as a signature of their mechanisms of action, Bates and her colleagues discovered that cytotoxic compounds could be identified on the basis of their vulnerability to extrusion by PGP. One of the identified compounds was a naturally occurring compound from *Chromobacterium violaceum*, which the Fujisawa Corporation (now Astellas Pharma) had identified as a potential cytotoxic agent, and had

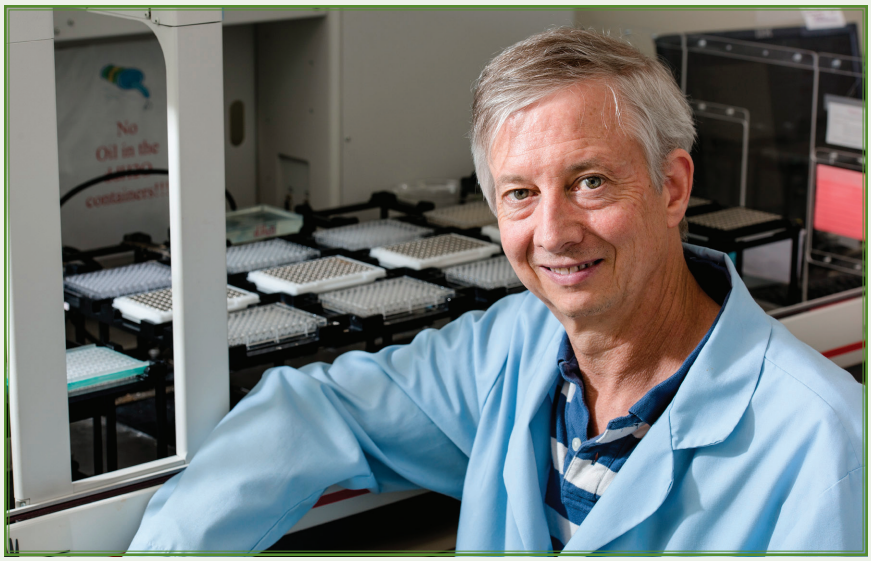
deposited with the Drug Screen. Bates and her colleagues at the Drug Screen and at NCI's Cancer Therapy Evaluation Program (CTEP) were intrigued enough by romidepsin's properties to initiate two phase 1 studies with the compound—one at NCI led by Bates.

In the lab, Bates and her NIH colleagues Dan Sackett, Ph.D., and April Robbins, Ph.D., found that the drug, romidepsin, promoted poor attachment of chromosomes to the kinetochore because of hyperacetylation in dividing cells, ultimately causing cell cycle arrest prior to mitosis, a mechanism which resembles the activity of the widely used chemotherapeutics, taxanes. Further preclinical work went on to show that romidepsin inhibits histone deacetylase (HDAC), thereby accounting for hyperacetylation and cell cycle arrest.

"We were trying it in solid tumors, because that is what I worked on, but we also enrolled a patient with T-cell lymphoma for this initial exploratory study. And his tumors just melted away," explained Bates. "When we saw the response in this patient, we quickly amended the study to add 10 more patients with T-cell lymphoma."

A phase 2 study soon followed, focused on both forms of T-cell lymphoma, which became a multi-institutional trial in 2001. In 2003, Bates presented their data to Richard Klausner, M.D., who was the NCI Director at that time, and to Klausner's visitor from Harvard, Greg Verdine, Ph.D. Verdine returned to Boston and, shortly thereafter, formed a company called Gloucester Pharmaceuticals to license the drug.

With exciting results mounting, CTEP was opening multiple clinical trials of romidepsin. And then, suddenly, several patients died. "We had two patients die in their sleep a few days after drug delivery," said Bates. Rather than stop the trial, Bates, Richard Piekarz, M.D., Ph.D., and other investigators at CTEP realized that the patients who had died had been at high risk for cardiac problems. Given the NIH Clinical Center's resources, they were able to augment the trial with close monitoring of EKG, cardiac tests, and supplemental potassium and magnesium.



(Photo: R. Frederickson)

Michael Dean, Ph.D.

"Then, we just kept accruing patients to the study," said Bates. Based on the phase 2 results from the studies performed by NCI and by Gloucester Pharmaceuticals, the U.S. Food and Drug Administration (FDA) approved romidepsin for the treatment of cutaneous T-cell lymphoma in 2009 and gave an accelerated approval for peripheral T-cell lymphoma in 2011. "I don't think it could have happened without CCR, because of the intensive safety data collection involved," said Bates.

"It's a very potent drug. We see prolonged responses, lasting years and years. One patient has been out of the trial for more than 10 years without relapse," said Bates. The overall response rate is a little over one third in the relapsed refractory setting for both indications. Ongoing and planned clinical trials are testing combinations of romidepsin with other targeted agents as well as radiation therapy.

A New Target

"When I first came to NCI in the 1980s, for a postdoctoral fellowship with George Vande Woude, his laboratory was characterizing a newly discovered gene that could transform cells in culture," said Michael Dean, Ph.D., Deputy Program Director of CCR's Cancer and Inflammation Program. The gene—*c-MET*—was isolated from human cell lines that had been

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chemically transformed *in vitro*. At the time, finding oncogenes meant transfecting mouse cells with pieces of DNA and assessing their transformation in culture and their ability to grow as implanted tumors.

"I generated some of the first DNA sequences for the gene and showed that it was homologous to other tyrosine kinases, some of which were known to be oncogenes." Dean and his colleagues mapped the gene to its chromosome and characterized the exact mutation that gave MET its transforming potential.

Following up on the role of *c-MET* in human cancers, the laboratory showed in the 1990s with Bert Zbar, M.D., some inherited kidney cancers were associated with germline mutations in the *MET* gene, and that tumors in these patients had increased copies of the mutant allele.

Mutations in c-MET are now found in a variety of tumors, and its mutations usually signal a poor disease prognosis. The gene encodes a receptor for hepatic growth factor/scattering factor (HGF/SF), which normally promotes cellular growth and proliferation during organ development, regeneration, and wound healing.

As a result, many cancer therapeutics companies have been interested in developing MET inhibitors. In 2012, the first of these inhibitors, Exelixis' Cometriq (cabozantinib), was approved for the treatment of metastatic medullary thyroid cancer.

"When we first started working on MET, we had no idea how long it would take to develop a drug. The development of Gleevec (imatinib) to treat chronic myelogenous leukemia in the late 1990s showed that it was possible to target oncogenic tyrosine kinases by targeting the ATP-binding domain in such a way that it shuts down the protein and isn't toxic." Once the principle was established, several companies joined the search for tyrosine kinase inhibitors as anticancer agents.

Gleevec has since been found to affect more than just its target protein; cabozantinib seems likewise to affect multiple signaling pathways. "There's a lot we don't understand about the biology of tumors and the importance of a single mutation," said Dean. "Once a drug is approved by the FDA, new uses will be discovered for that drug that we can't even envision."

Contingency Plan

Methotrexate, an inhibitor of folic acid metabolism, is a powerful chemotherapeutic agent, used for decades in the treatment of a broad spectrum of cancers. For a doubly unlucky 1.5–2 percent of patients treated with the drug, high-dose methotrexate administration can also lead to life-threatening toxicity. Usually given safely in very high doses, it can occasionally precipitate and lead to acute kidney dysfunction, which results from sustained exposure to very high drug concentrations.

When Brigitte Widemann, M.D., now a Senior Investigator in CCR's Pediatric Oncology Branch, began working as a fellow with David Poplack, M.D., and Peter Adamson, M.D., at NCI, they had shown in non-human primates that the bacterial enzyme carboxypeptidase-G2 (now glucarpidase) very rapidly reduced plasma concentrations of methotrexate. "With the data from monkeys, we developed a clinical trial to give the enzyme to patients who had high-dose methotrexate-induced kidney dysfunction," explained Widemann. The trial began with just a few patients in 1992; by the end, more than 300 patients were enrolled from across the U.S. and Europe.

"We were on call 24/7, as were the CTEP pharmacists," said Widemann. "We'd assess the patient information, talk to the physicians, and ship the drug to wherever it was needed. Blood samples were sent back to us for evaluation in real time. Within 15 minutes of administration of the enzyme, we would see an almost two-log decrease in methotrexate concentrations in every single patient."

Critically, by working with the FDA, the researchers were able to successfully make the case that plasma methotrexate levels were a good surrogate for the development of toxicity. "Ideally, you want to give this to patients with very high plasma methotrexate concentrations before they developed any clinical toxicities." Of the patients that

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(Photo: R. Baer)

Brigitte Widemann, M.D., Michelle O'Brien, R.N., a Research Nurse who worked on the clinical trials for glucarpidase, and Nalini Jayaprakash, M.S.

received the enzyme within 48 hours of starting their methotrexate infusion, no fatalities were reported. But patients who received the enzyme at later time points after the drug was already distributed into the cells were often not so lucky.

However, by the time the healthcare company BTG sought approval for the drug, the availability of new FDA guidelines required additional work prior to seeking approval. "The drug was initially not manufactured according to current FDA requirements and the high performance liquid chromatography (HPLC) assays for methotrexate concentration had to be revalidated using the now available FDA guidance." Fortunately, Nalini Jayaprakash, M.S., who began working with David Pollack's team in 1991, and had come to NCI from the pharmaceutical industry was able to revalidate the assay using the new FDA guidelines and compared old and new lots of the enzyme to satisfy regulators; meanwhile, the team ran a 30-patient prospective trial to confirm their earlier findings. Glucarpidase is now approved under the trade name Voraxaze, for the treatment of toxic plasma methotrexate concentrations.

"The FDA came to do an audit on our data and we learned how important meticulous documentation and standard operating procedures are for regulatory approval," said Widemann. "If you want to develop a drug, you have to be very patient and persistent. Ours was a real team effort within the laboratory, CTEP, and across agencies, and I am very happy to be part of bringing a life-saving drug to the clinic."

To learn more about Dr. Bates's research, please visit her CCR website at https://ccr.cancer.gov/susan-bates.

To learn more about Dr. Dean's research, please visit his CCR website at https://ccr.cancer.gov/michael-dean.

To learn more about Dr. Lowy's research, please visit his CCR website at https://ccr.cancer.gov/douglas-r-lowy.

To learn more about Dr. Schiller's research, please visit his CCR website at https://ccr.cancer.gov/john-t-schiller.

To learn more about Dr. Widemann's research, please visit her CCR website at https://ccr.cancer.gov/brigitte-c-widemann.